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CYTOSTATIC GENE TRANSFER FOR VASCULAR PROLIFERATIVE DISORDERS

Clinically significant restenosis remains the major complication of all percutaneous coronary revascularization procedures, occurring in 30% to 50% of patients within the first 6 months after revascularization. Although a number of factors contribute to restenosis, smooth muscle cell migration and proliferation and extracellular matrix deposition appear to be important pathophysiologic mechanisms. More than 30 different drugs and devices have been used in an attempt to prevent restenosis. This presentation outlines recent progress in using cytotoxic and cytostatic gene therapy for the prevention of restenosis. Recent work suggests that catheter-mediated delivery of replication-defective adenoviruses can be used to efficiently transduce medial vascular smooth muscle cells (VSMCs) after balloon angioplasty. Replication-defective adenoviruses encoding a nonphosphorylatable, constituitively active form of the retinoblastoma gene (AdΔRb) and the herpes simplex virus thymidine kinase gene (AdvTk) were constructed. Infection of primary rat aortic smooth muscle cells with AdΔRb completely inhibited growth factor–stimulated VSMC proliferation in vitro. More importantly, AdΔRb infection of balloon-injured arterial segments reduced restenosis by 50% to 70% in the rat carotid and porcine iliofemoral models of balloon angioplasty. Safety studies demonstrated (1) no vascular inflammation, (2) no evidence of distant infection with AdΔRb after local catheter delivery, and (3) no significant abnormalities of serum chemical composition or clotting parameters. Finally, infection with AdΔRb did not inhibit reendothelialization of the balloon-injured vessels. In a parallel set of experiments, infection of primary rat aortic smooth muscle cells with the AdvTK vector followed by ganciclovir treatment resulted in the efficient killing of proliferating cultured vascular smooth muscle cells in vitro. More importantly, infection with this vector followed by systemic ganciclovir treatment resulted in 50% to 70% reductions in restenosis in both the rat carotid and porcine iliofemoral artery models of balloon angioplasty. There were no local or systemic toxicities associated with AdvTk plus ganciclovir therapy. In summary, catheter-mediated delivery of adenovirus vectors represents an efficient means of delivering recombinant genes to vascular smooth muscle cells at the site of balloon angioplasty. Both cytotoxic and cytostatic genes have been used to significantly reduce restenosis in two animal models of balloon angioplasty. Gene therapy holds promise as a safe and effective means of inhibiting restenosis after balloon angioplasty and other percutaneous revascularization procedures in human beings.

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MODIFICATION OF THE VASCULAR PHENOTYPE BY VIRAL GENE TRANSFER: THERAPY AND TOXICITY

Genetic modification of the vessel wall holds promise for the study of vascular pathophysiology and for the therapy of vascular disease. Using a variety of techniques, including viral and liposome-mediated gene transfer, specific genes may be introduced into vascular smooth muscle cells or endothelial cells to modify the vascular phenotype. Eventually such an approach may be clinically applied to artery or vein grafts, mechanically denuded vessels, or prosthetic grafts in the operative setting to prevent thrombosis,

restenosis, or recurrent disease. Work in our laboratory has focused on using gene transfer to increase the production of fibrinolytic proteins by endothelial cells on seeded prosthetic grafts, and to locally deliver the potent thrombin inhibitor, hirudin, to the wall of an injured artery.

Genetic manipulation to decrease the thrombogenicity of a vascular segment is an attractive goal because early postoperative thrombosis is a frequent cause of arterial bypass failure. To enhance vascular antithrombotic activity, endothelial cells might be genetically modified to overexpress fibrinolytic molecules such as urokinase or t-PA. Modified endothelial cells might then be used to seed prosthetic grafts or denuded vessel surfaces to prevent thrombotic complications. In addition, endothelial cells within vascular segments may be transduced in vivo or autologous vascular grafts may be modified ex vivo to diminish their thrombotic potential.

In initial work we reported that retrovirus-mediated transfer of t-PA or urokinase to sheep as well as nonhuman primate endothelial cells in vitro resulted in increased plasminogen activator activity.[1,2] Implantation of collagen-coated vascular graft segments seeded with these genetically engineered endothelial cells reduced platelet and fibrin deposition in a baboon model of thrombosis of an arteriovenous shunt.[3] Importantly, the measured enhancement of local fibrinolysis occurred without an increase in systemic fibrinolytic activity.

Although autologous artery or vein is the conduit of choice for peripheral and coronary artery revascularization procedures, the limited availability of these vessels has stimulated tremendous interest in the development of prosthetic grafts with equivalent patency rates. A potential clinical application of the vascular graft seeding protocol used in the baboon shunt experiment described above[3] would be to coat small-diameter prosthetic grafts with endothelial cells. In this manner early thrombotic occlusion might be prevented. Several attempts to create grafts lined with endothelial cell monolayers, however, have produced only variable success in improving long-term patency rates, and no success has been reported in applying seeding technology to small-diameter (<6 mm) prosthetic grafts. To explore the possibility that small-diameter graft patency rates might be improved by seeding the grafts with fibrinolytically "enhanced" cells, we seeded 4-mm diameter collagen-impregnated Dacron grafts with endothelial cells engineered to overexpress t-PA. The seeded grafts were implanted both in vitro in a flow simulator and in vivo as interposition grafts in the common femoral or common carotid arteries of sheep.[4] Unfortunately, we found that endothelial cells overexpressing t-PA were retained at low rates both in vitro and in vivo, when compared with endothelial cells engineered with a marker gene, β-galactosidase. Control experiments suggested that the observed low retention was due to increased proteolytic activity produced by the enzymatically active t-PA. Thus not only did t-PA promote thrombolysis, it also promoted cell detachment. One potential solution to this problem might involve the development of a graft matrix component that is resistant to proteolysis, thereby improving endothelial cell retention. Alternatively, modifying endothelial cells to produce other proteins such as hirudin (which inhibit thrombosis but lack proteolytic activity) may result in improved endothelial cell adherence to grafts while also increasing graft patency rates. These hypotheses await testing; at present the utility of genetic engineering to increase small-diameter graft patency rates remains unproven.

Retroviral vectors have been used by several groups to transfer genes to vascular cells in vitro. However, the utility of retroviral vectors for in vivo vascular gene transfer is limited, due both to low titers and to the inability of these vectors to transfer genes into nondividing cells. In contrast, adenoviral vectors are notably successful for in vivo vascular gene transfer.[5] Adenoviral vectors may be propagated and concentrated to very high titers and can efficiently transfer genes into nondividing cells, such as the endothelial and smooth muscle cells of a quiescent artery wall. We have used adenoviral vectors to transfer genes to endothelial and smooth muscle cells of sheep, rat, and rabbit arteries in vivo.[6-8] In vivo gene transfer efficiency is reliably high: 20% to 30% of cells exposed to the vectors are transduced and significant quantities of recombinant proteins are produced in vivo after adenovirus-mediated gene transfer. These quantities (13 to 550 ng recombinant protein/mg vessel wall protein) appear adequate to alter the arterial phenotype, as they are comparable with levels of endogenous arterial proteins such as t-PA and basic fibroblast growth factor.[9] Disadvantages of adenoviral vectors include short-term gene expression and both acute and chronic host inflammatory responses following adenovirus infection. Short-term expression, however, might be an advantage depending on the therapeutic goal. For example, preventing thrombus formation on a denuded vessel surface after endarterectomy may require only transient gene expression until reendothelialization has taken place. Furthermore, improved adenoviral vectors that cause less inflammation may soon become available.[10]

After our initial dosing and toxicity studies we constructed an adenoviral vector capable of expressing recombinant hirudin.[11] Hirudin is an extremely potent and specific inhibitor of thrombin, and expression of hirudin in an artery wall would be expected to reduce local thrombin activity. Thrombin is thought to play an integral role in neointima formation after arterial injury,[12] and inhibition of thrombin by hirudin could therefore decrease neointima formation. Local delivery of hirudin has potential advantages over systemic delivery in that with local delivery, systemic anticoagulation and hemorrhage might be avoided.

Adenovirus-mediated gene transfer of hirudin to balloon-injured carotid arteries resulted in the secretion of biologically active hirudin by rat vascular smooth muscle cells and decreased neointima formation.[11] Importantly, local hirudin delivery did not affect systemic partial thromboplastin times. This study demonstrated that in vivo gene transfer could be used to modify the local vascular phenotype without causing undesirable systemic effects.

Although adenoviral vectors can efficiently transfer genes to the vessel wall in vivo, their utility might be limited by inflammatory responses evoked by adenovirus infection. Delivery of highly concentrated virus to the artery wall can produce both cellular toxicity and an acute inflammatory response.[7] Expression of open reading frames encoded within current generation adenovirus vectors may elicit both humoral and cellular immune responses.[10] Because production of arterial inflammation after adenovirus-mediated gene transfer could create rather than ameliorate vascular disease, we investigated the effects of exposure of normal arteries to replication-defective adenovirus vectors.[8] Exposure of rabbit femoral arteries to a replication-defective adenovirus vector provoked: (1) a significant vascular inflammatory response characterized by pronounced T lymphocyte infiltration throughout the artery wall; (2) up-regulation of both ICAM-1 and VCAM-1 expression by vascular smooth muscle cells; and (3) neointimal hyperplasia. Therefore, current adenovirus vectors are not merely benign delivery vehicles for the expression of transgenes in the arterial wall. The current utility of adenovirus vectors both for biologic and therapeutic studies is limited by the inflammatory response that results from the host response to vector proteins.

Our laboratory is currently pursuing two approaches to circumvent the inflammatory response to adenovirus: (1) suppression of the immune response to viral protein expression by immunomodulation of the host; and (2) development of new adenoviral vectors that are less immunogenic. We are currently

testing both of these approaches in our models of arterial gene transfer.

In conclusion, vascular gene transfer has the potential to produce profound local biologic effects without perturbing systemic physiology. In this role, gene transfer may eventually become a clinically useful targeted therapy for specific types of vascular disease. In 1996, however, substantial issues of efficiency, duration of expression, toxicity, and long-term efficacy remain to be addressed. The promise of viral gene therapy in the treatment of vascular disease is only now beginning to be clarified.

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DIRECT TRANSFER— NONVIRAL APPROACHES

ecNOS AND INTIMAL HYPERPLASIA

We have adopted a broad view of gene therapy as part of the emerging therapeutic armamentarium of molecular medicine in which DNA is used as a therapeutic agent of "drug" capable of modifying biologic or pathologic processes to ameliorate the course of disease. Accordingly, our objectives have been (1) to use the technology of genetic engineering to elucidate the fundamental molecular mechanisms of cardiovascular disease and (2) to use DNA transfer technology to modify these pathobiologic processes in an attempt to develop novel therapeutic strategies that alter the natural history of cardiovascular disease.

Although there are many strategies for modifying gene expression in the intact animal, we have used modified liposomes as molecular delivery systems. In particular, we have adopted the use of Sendai virus hemagglutinating virus of Japan (HVJ) conjugated to liposomes as a highly efficient method for introducing expression vector plasmids and oligonucleotides into cardiovascular tissues. We have successfully used this transfection system to modify gene expression within the vasculature, the kidney, the heart, and the liver. The efficacy of the HVJ-liposome system as a molecular delivery system will be discussed. This system may serve as a prototype of a molecular delivery system in which the proteins expressed by viruses that ordinarily facilitate cellular uptake are separated from the viral genome and employed in artificial DNA delivery complexes. We have successfully used this molecular delivery system to modulate vascular gene expression with antigene strategies such as antisense oligonucleotides or transcription factor cis element decoys as well as gene augmentation approaches using plasmid expression vectors.

Our discussion will focus on several in vivo genetic engineering strategies that we have used to modify cell proliferation in the vasculature and the kidney, which may alter the natural history of vascular and glomerular diseases. We are intrigued by the observation that acute manipulation of gene expression within the vessel wall that arrests cell-cycle progression has long-term consequences on vascular function and structure. In particular, we will highlight the potential use of these genetic engineering strategies to create bioengineered vein grafts that are resistant to atherosclerosis. In further characterization of this intriguing phenomenon, we have observed that the long-term efficacy of our genetic engineering strategy in conferring antiatherogenic properties to the vein grafts is associated with the preservation of normal endothelial function as evidenced by nitric oxide generation. We are currently characterizing the molecular basis of this phenomenon.

Many studies have documented the association between vascular disease and endothelial dysfunction; however, it remained to be demonstrated whether nitric oxide is actually an endogenous inhibitor of vascular lesion formation in vivo. We directly tested this hypothesis by transfecting the endothelial cell–type nitric oxide synthase III gene into the vessel wall after balloon angioplasty injury as a gene therapy strategy to prevent restenosis. These studies provided the first direct evidence that nitric oxide inhibits vascular lesion formation in vivo.

Although there are a number of potential gene therapy strategies for the treatment of vascular disease, there is compelling evidence that the nitric oxide genes fulfill many of the characteristics of an ideal candidate. An important limitation of current approaches to modulate gene expression within the vessel wall is the relatively low efficiency of all of the gene transfer methods used to date. The majority of cells within the vessel wall fail to express the gene therapy target gene. Therefore, gene therapy strategies that